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Comprehensive Analysis: Phytochemical Screening and Antioxidant Activity of Bombax ceiba and Camellia sinensis

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Abstract

This study presents a thorough examination of the phytochemical composition and antioxidant activity of *Bombax ceiba* and *Camellia sinensis*. Through extensive phytochemical screening, we identify and analyze the diverse bioactive compounds present in both plants. The physiological study delves into the intricate details of the plants physiological attributes, shedding light on their unique characteristics. Furthermore, the antioxidant activity is meticulously assessed to gauge the potential health benefits associated with these plant extracts. This study focuses on the determination of the total flavonoid content and DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity of selected plant extracts. The total flavonoid content is assessed using a standardized method, providing insights into the concentration of flavonoids in the extracts. The total phenolic content is assessed for their traditional therapeutic uses by utilizing specific method or technique for the extraction and quantification of phenolic compounds. The DPPH scavenging activity is evaluated to understand the antioxidant potential of the plant extracts. The abstract summarizes the methodology and key findings related to the total flavonoid content and antioxidant activity, offering a concise overview of the study's contributions to the understanding of the medicinal properties of *Bombax ceiba* and *Camellia sinensis*.

Keywords: Bombax ceiba, Camellia sinensis, DPPH scavenging activity, Flavonoid content, Phenolic content

Introduction:

India, renowned as the "Botanical Garden of the world," boasts an extensive array of medicinal plants. These plants have not only played a crucial role in shaping material medica but also stand as nature's bestowed gift to foster disease-free, healthy living, thereby contributing significantly to the preservation of human health. India, characterized by immense medico-cultural diversity, upholds a time-honored tradition where the medicinal plant sector remains integral and revered. Traditional systems of medicine, such as Ayurveda, Unani, and Siddha, thrive within this cultural tapestry.

Plants, as potent biochemists, have been pivotal components of phytomedicine since time immemorial, providing a diverse array of industrial chemicals. The bioactive constituents derived from plants—be it bark, leaves, flowers, roots, fruits, or seeds—underscore the versatility of natural sources in medicine. (Saric et al 2016)

Natural products constitute a vital wellspring of novel compounds with applications across major disease areas. These compounds, honed by evolution, exhibit an intrinsic ability to interact with proteins and other molecules. Approximately half of today's medicinal arsenal originates from natural sources, underlining their ongoing significance.

The future holds promising prospects for higher plants as sources of medicinal agents for research, prevention, and treatment of diseases. Despite the wealth of plant species—estimated at 250,000-400,000—only a meager 6% have undergone biological activity studies, and merely 15% have been subjected to phytochemical investigations. This underscores the imperative for systematic, activity-guided phyto-pharmacological evaluations of herbal drugs to unlock the untapped potential within nature's pharmacopeia. (Chaudhary and Tawar 2019)

Polyphenols, inherent compounds in plants and prevalent in everyday foods like nuts, fruits, vegetables, chocolate, wine, and tea, naturally occur. Following ingestion, these polyphenols are absorbed in the intestinal epithelium, demonstrating their ability to enter systemic circulation. Functioning as antioxidants, polyphenols actively contribute to averting oxidative damage induced by reactive oxygen species (ROS). Their notable anti-inflammatory and anti-carcinogenic properties suggest potential in preventing cardiovascular disease. Additionally, polyphenols may serve as a protective barrier against ultraviolet radiation (UVR) to safeguard the skin.(Jamdar and Shaikh 2017)

Bombax ceiba, commonly referred to as the Silk Cotton Tree, holds significance as a vital medicinal plant in tropical and subtropical regions of India. This towering deciduous tree features a straight buttressed trunk and expansive spreading branches. (Gurunani and Karadi 2018) According to Ayurveda, Bombax ceiba is attributed with well-established medicinal properties, making it a key ingredient in various formulations. Virtually every part of this plant is utilized for medicinal purposes, with a particular emphasis on the roots and flowers, which are employed in treating a diverse range of ailments. (Karole 2017)

Tea is most widely consumed beverage globally and serves as a significant supplier of plant polyphenols in human dietary intake. The plant responsible for this beverage variety is Camellia sinensis, which yields different types of teas based on specific processing methods.(Mendekar et al 2017) Green tea, derived from fresh leaves, is crafted to prevent the oxidation of polyphenolic components, primarily catechins. (Saric et al 2016)

This article seeks to present a comprehensive overview of both Bombax ceiba and Camellia sinensis, encompassing preliminary phytochemical screening, physiological studies, and antioxidant investigations.

Material and metohods

Camellia sinensis Leaves and ***Bombax ceiba*** thorns were shed dried. Dried Leaves and dried thorns were subjected to size reduction to obtain a coarse powder.

Preliminary evaluation of the crude drug

Organoleptic Evaluation

This includes the evaluation of drugs by sensory organs (skin, eyes, tongue, nose, ears) or gross evaluation. This includes evaluating drugs according to characteristics such as color, odor, taste, size, shape, and texture. Sensory and macroscopic evaluations of the *Camellia sinensis* and *Bombax Ceiba* Thorns were performed to study the morphological and sensory profiles of the herb**.** Organoleptic evaluation of thorns and leaves are given in table no. 1

Table no. 1 Organoleptic Evaluation

Parameter	***Camellia Sinensis***	*Bombax ceiba*
Colour	Dark Green	Reddish brown
Odour	Herbal aromatic	Characteristic
Taste	Bitter	-
Size and shape	Lanceolate	Sharp Conical

Physical Evaluation
Camellia sinensis leaves and *Bombax ceiba* thorns powder were carried out to evaluate parameters such as total ash value, acid insoluble ash, water soluble ash, alcohol soluble extracts, water-soluble extracts and extraction values such as loss on drying decided.

1. Loss on drying (Moisture Content):
Water and volatile materials in the crude drug are both identified by the test for loss on drying. When it is known that the herbal ingredients are hygroscopic, the loss on the drying test is crucial. Herbal materials with too much water will promote microbial growth, the presence of fungi, insects, and degradation. The water content gives information about the quality and shelf life of the medications in modern pharmaceutical technology. The mass loss after drying is measured as % w/w.(Neware et al 2022)

Procedure:
About 5 g of the drug was weighed in a porcelain dish and dried at 105°C for 5 hours in the oven. Then it is cooled and weighed. The loss of weight is recorded as moisture content

Ash Values

A. Determination of Total ash value
An exact weight of 2 g of powdered drug raw material was placed in a weighed crucible. At first, it was burned gently, then the temperature was gradually increased until it was free of carbon and cooled. The resulting ash was weighed and the percentage of total ash for air-dried crude drug samples was calculated.

B. Determination of Acid – insoluble ash value
The ash obtained in step (A) was boiled with 25 ml of 2M hydrochloric acid for 5 minutes. Insoluble matter was collected in a crucible. Washed with hot water and ignited. Then let it cool and weigh. The percentage of acid-insoluble ash was calculated based on the dried drug according to the formula.

Preparation of 2M hydrochloric acid solution

Hydrochloric acid (2 M) was prepared by diluting 17 mL of concentrated hydrochloric acid solution to 100 mL with water in a volumetric flask.

C. Determination of Water – soluble ash value
The ash obtained in step (A) was boiled in 25 ml of water for 5 minutes. The insoluble was collected, washed with hot water and ignited for 15 minutes. The percentage of water-soluble ash was calculated on a dried basis.

Extractive Value

A. Determination of Alcohol-soluble extractives
Accurately weighed 5 g of powder was macerated with 100 ml of 95% ethanol for 24 hours and filtered. 25 ml of the filtrate was evaporated to dryness in a tared porcelain dish and weighed. Per cent ethanol soluble extract values were calculated concerning air-dried drug according to the formula.

B. Determination of Water–soluble extractives
Water-soluble extracts were measured using the same procedure as alcohol-soluble extracts, using chloroform water instead of ethanol as solvent.

Preparation of Chloroform water
About 2.5 mL of chloroform was shaken with 900 mL of water and then diluted to 1000 mL with water.

Extraction of the plant materials

Maceration.

Camellia sinensis and Bombax ceiba

Extraction was done by the maceration process. 500gm tea leaves coarse powder was weighed then Ethanol & water (50:50) is poured on top until completely covered the drug material. (Koch et al 2019) 500gm ***Bombax ceiba*** thorns coarse powder was weighed then Ethanol & water (80:20) was poured on top until the drug material was completely covered. The container is then closed and kept for at least seven days. Filtration was done using Muslin cloth and solvent was removed using a vacuum oven. Dried extracts were stored at 4^{o}C till use. (Miyata et al 2018)

Solubility

A. Solubility of *Camellia sinensis and Bombax ceiba* extract:

The solubility of ***Camellia sinensis and Bombax ceiba*** extract in various oils (Isopropyl myristate, Captex 35, Oleic acid, and Capmul 708G, Corn oil), surfactants (Tween 20, Brij 35 and Tween 80), and cosurfactants (Propylene glycol, Transcutol HP) was determined by dissolving a 100 mg extract in 1 gm of each of the selected oils, surfactants, and cosurfactants in stoppered vials. The mixtures were continuously stirred using a vortex mixer for 10 min. solubility was determined visually.

Phytochemical Screening of *Camellia Sinensis and Bombax Ceiba*

The hydroalcoholic extract was tested for physicochemical constituents as per the following tests.

Preparation of extract solutions:

10 mg of dried extract was dissolved in ethanol and the volume was made up to 10 ml with Ethanol. These were subjected to chemical tests.

A) Detection of Flavonoids:

1. Alkaline reagent test:

To 2ml of test solution, 2ml of sodium hydroxide was added.

The appearance of yellow colour becomes colourless with the addition of diluted acid. Indicates the presence of flavonoids.

2. Lead acetate test:

To 2ml of test solution, a few drops of 10% lead acetate solution were added, Appearance of yellow precipitate, indicates the presence of flavonoids.

3. Ammonia test:

To 2ml of test solution, 5ml dil. Ammonia solution was added, Appearance of yellow colour. In addition to conc. H_2SO_4, Indicates the presence of flavonoids. (Barkat and Mahmood 2018)

4. Ferric chloride test:

2ml of test solution when treated with a few drops of 10% ferric chloride solution, gives a green precipitate Indicating the presence of flavonoids.

B) Detection of Phenolic compounds:

1. Iodine test:

To 2ml of test solution, a few drops of dil. Iodine solution was added, Appearance of transient red /yellow colour, indicating the presence of phenolic compounds.

2. Potassium dichromate test:

To 2ml of test solution, a few drops of potassium dichromate solution were added, and a dark red. The colour indicates the presence of flavonoids. (Chaudhary and Tawar 2019)

C) Tests for Tannins:

1. Ferric-chloride test:

2 ml of test solution treated with a few drops of ferric chloride solution. The development of dark green colour indicates the presence of tannins.

2. HCL test:
2ml 1% HCl was added to 2 ml of test solution, Appearance of red precipitate, indicates the presence of tannins.

D) Detection of reducing sugars:
1. Fehling's test:
1ml each of Fehling's solutions A & B were added to 1ml of the test solution and then boiled in a water bath. Red precipitate was not obtained indicating the absence of reducing sugars.

E) Detection of Glycosides
1. Baljet's test (Legal Test):
2 ml of the test solution was treated with 2 ml of sodium picrate solution. Yellow to orange color was not developed indicating the absence of cardiac glycosides.
2. Keller-Killiani test:
1.5ml glacial acetic acid was added to 1ml test solution then 1 drop of 5% ferric chloride and conc. H_2SO_4 (along the side of the test tube) was added, and blue coloured solution indicates the presence of glycoside.
3. Borntrager's test:
3ml Chloroform was added to 1ml test solution, shaken well, and 10% Ammonia solution added. The pink colour was not obtained.
F) Tests for Saponin:
Foam Test:
Powdered extract (10-20 mg) was shaken vigorously with water (1 ml). The persistent foam was developed. Indicates the presence of saponin.

G) Detection of Phytosterols
1. Salkowski's test:
To 1ml of test solution, a few drops of conc. H_2SO_4 was added and shaken well and allowed to stand, Form Red colour in the lower layer, indicates the presence of Phytosterols.
2. Liebermann Burchard's test:
The 0.5 ml of test solution was treated with 2 ml of acetic anhydride, mixed well and then 2 ml of concentrated sulfuric acid was added from the sides of the test tube. Colour change was not observed indicating the absence of Phytosterols.
3. Hesse's response:
To 5ml aq. extract, 2ml chloroform was added, and then 2ml conc.H_2SO_4 was added. The red colour was not observed indicating the absence of Phytosterols.

H) Detection of Triterpenoids:
1. Salkowski's test:
To 1ml of test solution, a few drops of conc. H_2SO_4 was added to the shaken well and allowed to stand, Golden yellow layer (at the bottom) was not formed, indicating the absence of Triterpenoids.

I) Detection of alkaloids:
1. Hager's test:
To 1 ml of test solution, 2 ml of Hager's reagent was added. The appearance of a yellow precipitate indicates the presence of alkaloids.
2. Mayer's test:
To the 1 ml of test solution, 2 drops of Mayer's reagent were added. The appearance of a yellow precipitate indicates the presence of alkaloids.
3. Wagner's test:
To the 1 ml of test solution, 2 drops of Wagner's reagent were added. The reddish precipitate was not observed indicating the absence of alkaloids.
4. Picric acid test:
To the 1 ml of test solution 3-4 drops of 2% picric acid solution were added. The orange colour was not observed indicating the absence of alkaloids.

J) Detection of Carbohydrates:
1. Molish's test:

2 drops of alcoholic α–naphthol were added to the 2 ml of test solution. Then 1 ml of concentrated Sulphuric acid was added slowly from the sides of the test tube. The Violet ring was not formed indicating the absence of carbohydrates.(Rasheed et al 2012)

2. Seliwanoff's Test:

3ml Seliwanoff's reagent was added to the 1 ml of the test solution and then heated in a water bath for 1 min. Rose red colour was not observed indicating the absence of carbohydrates.(Rani et al 2022)

3. Test for Starch:

5ml 5% KOH solution was added to the 1 ml of test solution. Cinery colour was not observed indicating the absence of starch.(Meena 2017)

4. Test for Pentoses:

2ml conc. HCL and little amount of phloroglucinol were added to the 2 ml of the test solution and then heated over a flame. The red colour was not observed indicating the absence of Pentose. All preliminary phytochemical screening of leaves and thorns extract are given in table no.3.

Table no.3 Preliminary Phytochemical screening of leaves and thorns

Constituents	***Camellia Sinensis*** **Leaves extract**	*Bombax ceiba* **Thorns extract**
Alkaloids	+	+
Carbohydrate	-	+
Reducing sugar	-	-
Phenolic compounds and Tannins	+	+
Flavonoids	+	+
Saponins	+	-
Steroids &Tri terpenoids	-	+
Glycoside	-	+

Determination of Total Phenolic Content:

Procedure:

Total phenolics were determined using the Folin-Ciocalteu reagent. A sample (1 ml) was mixed with 5 ml of Folin-Ciocalteu reagent and allowed to stand at room temperature for 6 min after which 4 ml of 7.5% Na_2CO_3 solution was added. (Li et al 2015)The solution was mixed and incubated at 45°C for 15 min. Plant extracts phenols are oxidized to a dark blue colour by the FCR reagent. The absorbance was measured at 760 nm. The results were calculated as mg of Gallic acid equivalent (GAE) per 1 g sample. Absorbance and concentration of gallic acid are given in table no. (Jafri et al 2022) (Phuyal et al 2020) Calibration curve of gallic acid given in fig. 1.

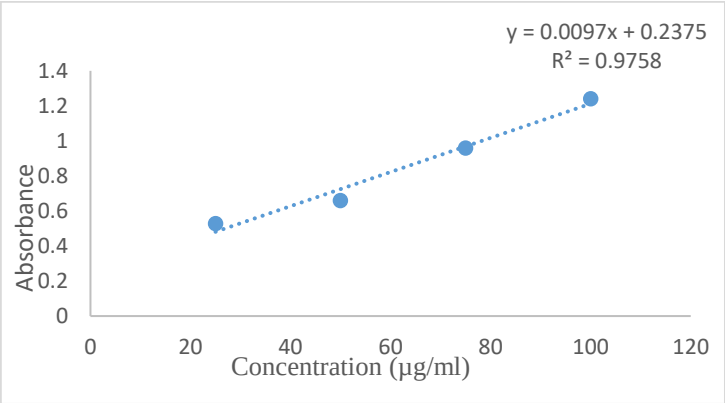

Fig.1 : Calibration curve for Gallic acid

Table no 4 : Concentration and absorbance for Gallic acid

Concentration (µg/ml)	Absorbance
25	0.528
50	0.659
75	0.96
100	1.24

Determination of total flavonoid contents:

Procedure:
A (1 ml) of the sample was mixed with 0.2 ml of 5% sodium nitrite After 5 min, 2 ml of 10% aluminium chloride was added to the mixture then 2 ml of 1 M sodium hydroxide was added. The solution was thoroughly mixed and the absorbance was measured at 510 nm. Total flavonoid content was expressed as mg Quercetin equivalents (QE) per 1 g sample given in tableno.5 and calibration curve for the same is given in fig.2.(Rani et al 2022)

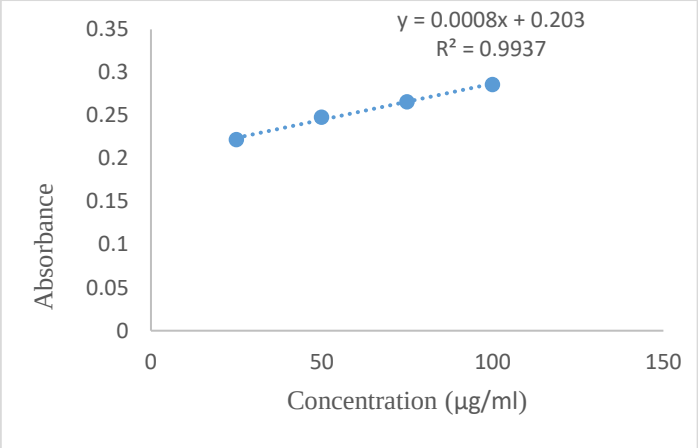

Fig. 2: Callibration curve for Quercetin

Table no 5 : Concentration and absorbance for Quercetin

Concentration (µg/ml)	Absorbance
25	0.222
50	0.248
75	0.266
100	0.286

DPPH free radical scavenging activity

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method is a widely used, quick, simple, and inexpensive method for measuring antioxidant capabilities that use free radicals to determine whether substances can produce hydrogen or act as free radical scavengers (FRS) (Guchu et al 2020) 0.8 mL of various extract/formulation concentrations were added to 1.2 mL of 0.1 mM DPPH solution, properly mixed and left in the dark for 30 minutes. Next, a UV-Visible Spectrophotometer was used to evaluate absorbance at 517 nm in comparison to ethanol. As a control, a solution for dilution mixed with DPPH in the same ratio as the sample was used.(Vishnoi et al 2018)
Using the following standard formula, the percentage inhibition of the DPPH solution by the test sample was determined:

% Inhibition = Absorbance of Control – Absorbance of sample/ Absorbance of Control*100

Results:

Physical Evaluation
The total ash value, water soluble ash value, acid insoluble ash value, and loss on drying were used to evaluate the crude drug. It is crucial for determining the purity and identity of the plant. Corresponding physical value of leaves and thorns are given in table no 2.

Table no.2 Physical Evaluation

Parameter	**leaves**	**thorns**
Total ash value	3.06	1.64
Acid insoluble ash value	4.11	8.536
Water soluble ash value	32.67	49.13
Loss on drying	8.6	11.3
Alcohol soluble extractive value	1.74	1.564
Water soluble extractive value	4.942	0.978

Preliminary Phytochemical Screening of leaves & Thorns

Camellia Sinensis consist of alkaloids, phenolic compounds, tannins, flavonoids, saponins whereas *Bombex ceiba* consist of alkaloids, carbohydrates, ,phenolic compounds, tannins, glycosides.

Total Phenolic Content:

Total Phenolic Content in *Camellia sinensis* extract was found to be 0.06322mg Gallic acid equivalent (GAE) per 1 g sample. of (i.e.,100 µg/ml)

Total Phenolic Content in B*ombax ceiba* extract was found to be 0.08433mg Gallic acid equivalent (GAE) per 1 g sample of (i.e.,100 µg/ml)

Total flavonoid contents:

The total flavonoid Content in *Camellia sinensis* was found to be **0.04625mg** Quercetin equivalents (QE) per 1 g sample. (i.e.,100 µg/ml)

The total flavonoid Content in *Bombax ceiba* was found to be **0.0575mg** Quercetin equivalents (QE) per 1 g sample. (i.e.,50 µg/ml)

In-vitro Antioxidant Activity:

Absorbance of control was found to be 0.97.Absorbance, % Inhibition, IC 50 value for *Camellia Sinensis* Extract are given in table no.6. Absorbance, % Inhibition, IC 50 value for *Bombax ceiba* Extract are given in table no 7. Absorbance, % Inhibition, IC 50 value for a mixture of extract are given in table no 8.

Table no.6 : Absorbance, % Inhibition, IC 50 value for *Camellia sinensis* extract

Concentration (ppm)	Absorbance	% Inhibition	IC 50
20	0.331	58.5	
40	0.316	60.4	
60	0.248	68.9	
80	0.175	78.1	
100	0.119	85.1	3.1963 µM

Table no.7 : Absorbance, % Inhibition, IC 50 value for *Bombax ceiba* extract

Concentration (ppm)	Absorbance	% Inhibition	IC 50
20	0.595	25.3	
40	0.465	41.7	
60	0.327	59.0	
80	0.229	71.3	
100	0.158	80.2	52.12 µM

Table no.8 : Absorbance, % Inhibition, IC 50 value for a mixture of extract

Concentration (ppm)	Absorbance	% Inhibition	IC 50
20	0.357	55.2	
40	0.318	60.1	
60	0.206	74.1	
80	0.186	76.66	
100	0.164	79.42	1.243 µM

In the DPPH assay, the extract reduces a violet-coloured DPPH solution to a yellow- coloured product, in a concentration-dependent way. The lower the IC50 value, the better the substance at scavenging free radicals or inhibiting oxidation. Among CS Extract, BC Extract, CS + B Mixture of Extracts the mixture of Extracts has a low IC50 value i.e., better free radical scavenging activity.

Discussion

The plants were taken for their pharmacognostic, phytochemical and pharmacological evaluation. In preliminary phytochemical tests of the *Camellia Sinensis* extract presence of alkaloids, phenolic compounds and tannins, flavonoids and saponins was confirmed and in *Bombax ceiba* extract alkaloid, carbohydrates, phenolic compounds and tannins, flavonoids steroids & tri-terpenoids and glycoside was confirmed. (Rajbhar et al 2015)Total Phenolic Content in *Camellia sinensis* and *Bombax ceiba* extract was found to be 0.06322mg and 0.08433mg respectively, Gallic acid equivalent (GAE) per 1 g sample of (i.e.,100 µg/ml).The total flavonoid Content in *Camellia sinensis* and *Bombax ceiba* extract was found to be 0.04625mg and 0.0575mg respectively Quercetin equivalents (QE) per 1 g sample (i.e.,100 µg/ml). IC50 value of *Camellia Sinensis* and *Bombax ceiba* extract was found to be 3.1963 µM and 52.12 µM respectively. IC50 value of *Camellia Sinensis+ Bombax ceiba* (mixture of extracts) was found to be 1.243 µM . This shows that the free radical scavenging activity is in the following order: CS + BC Mixture of Extracts > CS Extract > BC Extract.

Conclusion

In conclusion, this comprehensive analysis of *Bombax ceiba* and *Camellia sinensis* has unveiled valuable insights into the phytochemical composition and antioxidant activity of these botanical specimens. The phytochemical screening revealed a diverse array of bioactive compounds in both plants, underscoring their potential therapeutic significance. The physiological study shed light on the unique physiological attributes of *Bombax ceiba* and *Camellia sinensis,* contributing to a deeper understanding of their distinct characteristics, particularly in relation to DPPH radicals. The observed antioxidant potential suggests that these plant extracts may hold promise in combating oxidative stress-related disorders and promoting overall health. The findings collectively emphasize the multifaceted nature of *Bombax ceiba* and *Camellia sinensis* as sources of bioactive compounds with potential applications in medicine and nutrition. This study not only contributes to the existing body of knowledge regarding these plants but also provides a foundation for future research endeavors. Further investigations into specific bioactive compounds, potential synergistic effects, and in vivo applications could offer more targeted insights, fostering the development of novel therapeutic interventions. Overall, the comprehensive analysis presented here serves as a valuable resource for researchers and practitioners in the fields of phytochemistry and medicinal plant research.

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